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Mass Spectrometry-Based Serum and Plasma Peptidome Profiling for Prediction of Treatment Outcome in Patients With Solid Malignancies

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. SELDI • MALDI • Cancer • Peptidomics • Personalized medicine • Blood

Abstract

Introduction. Treatment selection tools are needed to enhance the efficacy of targeted treatment in patients with solid malignancies. Providing a readout of aberrant signaling pathways and proteolytic events, mass spectrometry-based (MS-based) peptidomics enables identification of predictive biomarkers, whereas the serum or plasma peptidome may provide easily accessible signatures associated with response to treatment. In this systematic review, we evaluate MS-based peptide profiling in blood for prompt clinical implementation.

Methods. PubMed and Embase were searched for studies using a syntax based on the following hierarchy: (a) blood-based matrix-assisted or surface-enhanced laser desorption/ionization time-of-flight MS peptide profiling (b) in patients with solid malignancies (c) prior to initiation of any treatment modality, (d) with availability of outcome data.

Results. Thirty-eight studies were eligible for review; the majority were performed in patients with non-small cell lung cancer (NSCLC). Median classification prediction accuracy was 80% (range: 66%–93%) in 11 models from 14 studies reporting an MS-based classification model. A pooled analysis of 9 NSCLC studies revealed clinically significant median progression-free survival in patients classified as "poor outcome" and "good outcome" of 2.0 ± 1.06 months and 4.6 ± 1.60 months, respectively; median overall survival was also clinically significant at 4.01 ± 1.60 months and 10.52 ± 3.49 months, respectively.

Conclusion. Pretreatment MS-based serum and plasma peptidomics have shown promising results for prediction of treatment outcome in patients with solid tumors. Limited sample sizes and absence of signature validation in many studies have prohibited clinical implementation thus far. Our pooled analysis and recent results from the PROSE study indicate that this profiling approach enables treatment selection, but additional prospective studies are warranted.

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Implications for Practice: Treatment selection tools are needed to enhance the efficacy of treatment in patients with solid tumors. Mass spectrometry-based peptidomics enables identification of predictive biomarkers, whereas the serum or plasma peptidome may provide easily accessible signatures associated with response to treatment. This review discusses 38 studies on blood-based peptidomics performed before initiation of systemic and/or local treatment and includes a pooled analysis based on pretreatment outcome classification in patients with non-small cell lung cancer. This analysis and recent results from the PROSE study indicate that this profiling technique enables treatment selection in patients with cancer, but additional prospective studies are warranted.

Introduction

Since 2003, more than 20 targeted therapies have reached clinical approval for treatment of patients with advanced solid tumors [1, 2], but their often temporary effectiveness in a limited selection of patients as well as their significant toxicities emphasize the need for a clinically applicable strategy to predict efficacy of these agents. Currently, mutation status guides treatment in some tumor types, but actual response to molecular-guided treatment is not guaranteed [3–5]. Because responses to targeted therapies depend on their effect on downstream signaling activities in tumor tissue, mass spectrometry-based (MS-based) proteomics provides a potential tool for prediction of response to these drugs by recognizing changes in protein abundance and activating post-translational modifications and protein-protein interactions [6, 7]. Blood has been suggested to provide the ideal biological sample for profiling of these effects due to the accessibility of the serum and plasma peptidome, consisting of proteins and peptides released by tissue (including tumor tissue) as a result of proteolytic cascades [8–10].

More than 20,000 research articles on matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) and surface-enhanced laser desorption/ionization time-of-flight

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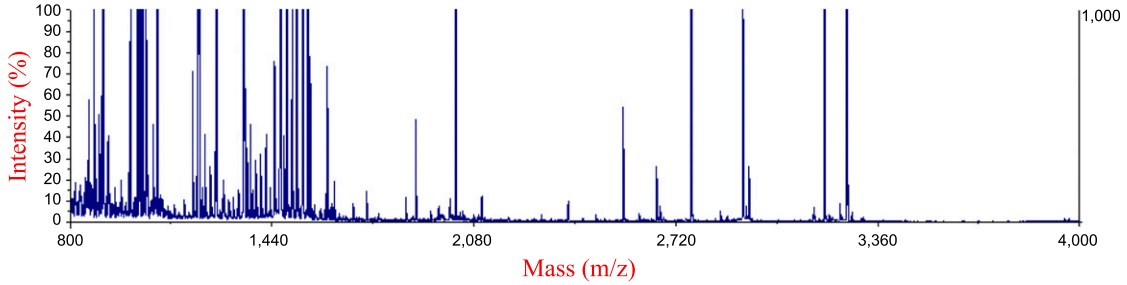

Figure 1. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry serum pattern. Representative example of a mass spectrum obtained from serum, showing several peptide peaks in the high- and low-intensity ranges.
Abbreviation: m/z, mass-to-charge ratio.

MS (SELDI-TOF MS) for profiling of the blood peptidome have been published since their development in the late 1980s and 1990s. Mass spectral peaks correspond to ions formed from peptides and proteins with a molecular weight of less than 20 kDa, and the peak amplitude indicates their abundance (Fig. 1) [11–13]. Several studies have reported on the potential for early detection by discriminating patients from healthy controls (HCs) based on differential serum proteome as a consequence of tumor biology [14–17], but clinical implementation has been hampered by the difficulties of other groups in reproducing the results [18, 19]. In the meantime, strict sample-handling procedures and unbiased study design have been shown to result in high intra- and inter-laboratory reproducibility of mass spectra and algorithm-based classification concordance [20–22]. In this paper, we have systematically reviewed studies on MS-based serum and plasma peptidomics performed in patients with solid tumors in relation to outcome following systemic and local treatment and evaluated its appropriateness for prompt implementation in the clinic.

Materials and Methods

Literature Search

Search engines PubMed and Embase were used to identify studies published until September 2013 using serum or plasma MALDI- or SELDI-TOF MS peptidome profiling in patients with solid malignancies prior to initiation of any treatment modality, with availability of outcome data including response and progression-free survival (PFS) or overall survival (OS). For PubMed, the following syntax was applied: ("Spectrometry, Mass, Matrix-Assisted Laser Desorption-Ionization"[MeSH] OR maldi[tiab] OR seldi[tiab] OR "surface enhanced laser desorption"[tiab] OR "matrix assisted laser desorption"[tiab] OR serum proteomic test) AND cancer[sb] AND ("Serum"[MeSH] OR serum[tiab] OR blood[subheading]). For Embase, the following syntax was used: "surface enhanced laser desorption ionization time of flight mass spectrometry"/exp OR "surface enhanced laser desorption ionization time of flight mass spectrometry" OR "matrix assisted laser desorption ionization time of flight mass spectrometry"/exp OR "matrix assisted laser desorption ionization time of flight mass spectrometry" OR "serum proteomic test":ab,ti OR seldi:ab,ti OR maldi:ab,ti OR "surface enhanced laser desorption":ab,ti OR "matrix assisted laser desorption":ab,ti AND ("serum"/exp OR "serum" OR serum:ab,ti OR "blood"/exp OR "blood") AND ("neoplasm"/exp OR "neoplasm"). Subsequent limits were applied for studies in humans and studies published in English since 1995, with an available abstract. Based on results of the syntax-based search, we performed an additional PubMed search using the search term "VeriStrat," a serum proteomic test.

Selection of Papers

Potentially relevant studies retrieved by the PubMed and Embase searches were independently reviewed for eligibility by three investigators (L.M.S., M.L., H.M.W.V.) and two investigators (M.L. and H.M.W.V.), respectively, according to aforementioned criteria. Unpublished studies were not considered eligible. Levels of evidence were not used to assess the value of each publication selected for inclusion.

Results

Number of Studies Meeting Selection Criteria

Using the PubMed and Embase syntaxes and aforementioned limits, 1,226 and 1,192 potentially relevant studies, respectively, were identified. Figure 2 depicts the subsequent stepwise selection of 37 eligible articles. With Embase, 74% of the PubMed-selected articles were found, but no additional eligible articles were found. One study that had not been identified by either syntax was found by an additional PubMed search for "VeriStrat," resulting in a total of 38 included articles for discussion in this review. The keywords of the non-syntax-identified article did not include SELDI, MALDI, serum, blood, or cancer.

Investigated Tumor Types and Treatment Modalities in Eligible Studies

Thirty of 38 eligible studies investigated blood-based peptidomics before systemic treatment, mainly in non-small cell lung cancer (NSCLC) and in breast cancer. In four of eight studies investigating local treatment, profiling was applied prior to chemoradiation (Fig. 3). Study details are summarized in Table 1.

Studies Reporting Profiling Prior to Systemic Therapy

Targeted Therapy in NSCLC

Twelve of 16 included studies investigating profiling prior to targeted therapy are related to the study by Taguchi et al. in patients with advanced NSCLC treated with tyrosine kinase inhibitors (TKIs) directed against epidermal growth factor receptor (EGFR) [21]. In this study, a training set of

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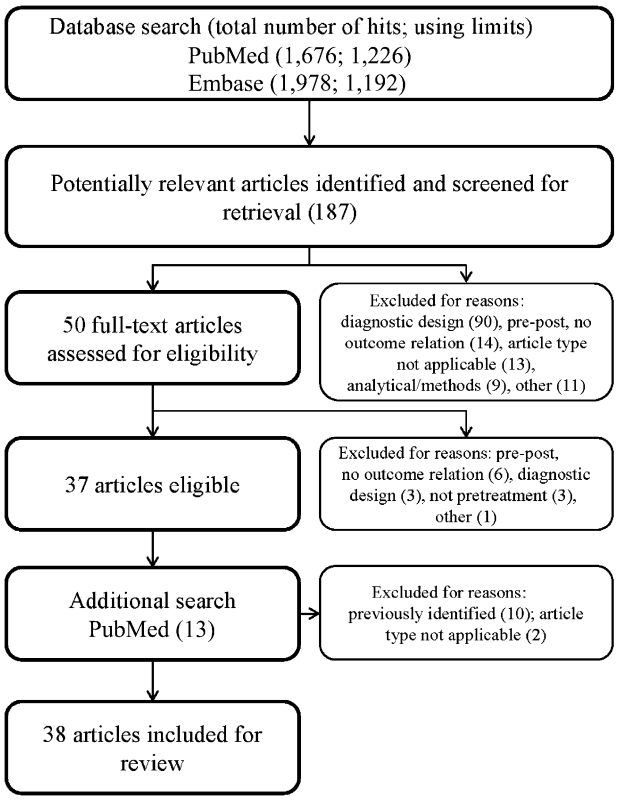

Figure 2. Database search and article selection. Search syntaxes were constructed in consultation with an information specialist. Screening of titles and abstracts was performed independently by three investigators. Full-text articles were independently appraised for predefined selection criteria for inclusion in the review.

pretreatment sera ($n = 139$, gefitinib) was supplemented with two validation cohorts ($n = 67$, gefitinib; $n = 96$, erlotinib; from ECOG-E3503) [23]. A control set of 158 patients who did not receive EGFR-targeting TKIs was included from three additional cohorts (2 advanced, 1 early stage). Eight differentially expressed mass-to-charge ratio (m/z) values or "peptide peaks" (5,843, 11,446, 11,530, 11,685, 11,759, 11,903, 12,452, and 12,580 Da) in the training set were used to construct an algorithm based on spectra from clinically most distinct patients in terms of time to progression (TTP) and OS. Patients with progressive disease (PD) within 1 month were classified as "poor outcome," and patients with stable disease (SD) for more than 6 months were classified as "good outcome." In the validation sets, TTP and OS were significantly longer in good-outcome patients compared with poor-outcome patients, with a median OS of 207 versus 92 days (hazard ratio [HR] of death: 0.50; $p = .054$) in the first validation cohort and 306 versus 107 days (HR: 0.41; $p < .001$) in the second validation cohort. No survival difference could be observed between both classifications in the control set. Interestingly, parallel application of the 8-peptide algorithm to plasma of 73 erlotinib-treated patients did not alter their serum-based outcome classification [21]. This algorithm has been commercialized as "VeriStrat" but will be further referred to in this article as the "Taguchi algorithm" (TA). Meanwhile, 12 additional studies have either applied or reported on the TA; 8 investigated nonoverlapping cohorts (Table 1).

Multivariate analysis by Amann et al. in 41 patients from the aforementioned erlotinib validation cohort [23] indicated that the algorithm predicted survival in patients with wild-type *EGFR* and independent of *KRAS* mutation status. However, the sample size of mutant tumors was small ($n = 12$) [24]. In 2012, Carbone et al. applied the TA to plasma profiles available for 441 of 731 patients from the randomized placebo-controlled BR.21 study [25], which established the role of erlotinib in patients with advanced NSCLC. Prognostic properties of the TA were confirmed in the placebo arm, showing superior median PFS and OS for good-outcome patients compared with patients classified as poor outcome (OS: 6.6 vs. 3.1 months; HR: 0.44; $p < .0001$). No significant correlation was found with *EGFR* or *KRAS* mutation status. Patients classified as good outcome seemed to benefit more from erlotinib than from placebo (OS: 10.5 vs. 6.6 months; HR: 0.63; $p = .002$), whereas OS for poor-outcome patients was not significantly different between arms (4.0 vs. 3.1 months; HR: 0.77; $p = .11$); the survival curves separated at times longer than 4 months. Multivariate adjusted analyses showed similar relative benefit from erlotinib for both classifications by a nonsignificant interacting p value, indicating a prognostic value of the algorithm. Nevertheless, erlotinib-treated patients classified as good outcome had a significantly higher response rate (RR) than poor-outcome patients (11.5% vs. 1.1%; $p = .002$) [26].

The TA has been applied to serum profiles of patients treated with erlotinib plus bevacizumab (erl/bev) in three studies. Analyzing 35 patient sera from a phase I/II study [27], Carbone et al. found median OS to be significantly longer for good-outcome patients (61 weeks) than for poor-outcome patients (24 weeks) (HR: 0.14, $p = .007$); median and PFS was also significantly longer at 24 weeks versus 8 weeks, respectively (HR: 0.045, $p = .003$). The TA classified all 8 patients with partial response (PR) and 6 of 7 patients with SD at more than 16 weeks as having good outcome, whereas 2 of 6 patients with initial PD were classified as poor outcome [28]. Gautschi et al. confirmed the superior median OS for good-outcome patients versus poor-outcome patients (13.4 vs. 6.2 months; HR: 0.48; $p = .003$) in 117 patients receiving first-line treatment with erl/bev. In contrast to the previous study, median PFS was not significantly different between good versus poor outcome classification (4.0 vs. 3.2 months; HR: 0.77, $p = .263$), suggesting, according to the investigators, that TA performance may be dependent on prior treatment [29]. This is supported by a phase II study by Akerley et al. with erl/bev in 42 previously untreated patients. Median PFS and OS were superior patients classified as good outcome compared with those classified as poor outcome (PFS: 18.9 vs. 6.3 weeks; $p = .0035$; OS: 71.4 vs. 19.9 weeks; HR: 0.27; $p = .0015$). PFS for study treatment plus subsequent chemotherapy was also significantly longer in good-outcome patients, underscoring the prognostic properties of the algorithm. Nonetheless, 9 of 10 observed responses, as assessed by the Response Evaluation Criteria in Solid Tumors (RECIST), occurred in patients with good-outcome classification [30]. Salmon et al. [31] developed an alternative 11-peak signature based on 37 erl/bev-treated patients [27]. Five peaks overlapped with the TA. In a validation set of 82 erlotinib-treated patients [23], compound scores from the MS features and their peak intensities could distinguish between patients with long and short PFS and OS,

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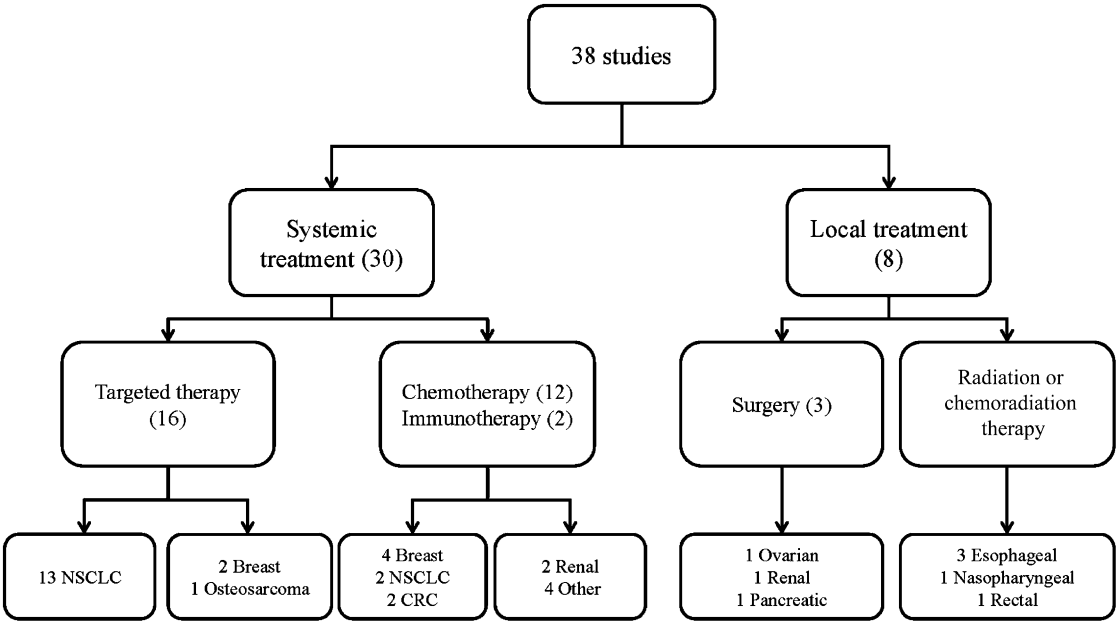

Figure 3. Tumor types and treatments in eligible studies. Overview of tumor types and treatment modalities in 38 eligible articles reporting matrix-assisted or surface-enhanced laser desorption/ionization time-of-flight mass spectrometry peptide profiling in pretreatment serum or plasma.

Abbreviations: CRC, colorectal cancer; NSCLC, non-small cell lung cancer.

discriminating between patients with compound scores lower than the median and equal to or greater than the median, whereas no significant difference was found in a control set of 61 chemotherapy-treated patients [31].

TA-based sorting of patient groups has been shown in the context of several other TKI-based treatment regimens. Analysis of TA performance by Kuiper et al. [32] in serum from 50 chemotherapy-naïve patients treated with erlotinib plus sorafenib [33] again confirmed superior outcomes for patients classified as good outcome versus poor outcome (OS: 13.7 vs. 5.6 months; HR: 0.30; $p = .009$; PFS: 5.5 vs. 2.7 months; HR: 0.40; $p = .035$), whereas the objective RR was not significantly different [32]. For sorafenib monotherapy, as reported by Dingemans et al. in 55 pretreated patients, PFS was significantly longer in TA-discerned good-outcome patients versus poor-outcome patients (2.6 vs. 1.5 months; HR: 1.4; $p = .029$), whereas OS was not significantly different (6.0 vs. 2.5 months; HR: 1.3; $p = .166$), probably due to a relatively large variation in OS duration [34]. Stinchcombe et al. applied the TA to sera of 98 elderly patients treated in a randomized phase II trial of first-line therapy with gemcitabine, erlotinib, or combination [64]. Superior median OS was observed for good-outcome patients versus poor-outcome patients in the erlotinib monotherapy arm only ($n = 32$; 255 vs. 51 days; HR: 0.40; $p = .014$), whereas PFS was significantly longer in good-outcome patients for both erlotinib-containing arms. The absence of prognostic power in the gemcitabine arm ($n = 28$) may have been due to crossing over to erlotinib after progression. Interestingly, a significant treatment interaction was demonstrated for patients in the monotherapy arms, indicating that good-outcome patients had more benefit from erlotinib, whereas poor-outcome patients benefited more from gemcitabine. In line with this observation, the authors point to the median PFS and OS of 22 and 51 days, respectively, in erlotinib-treated patients classified as poor outcome, suggesting that this drug is not an acceptable first-line treatment option for these patients [35].

Three additional studies have reported results of profiling prior to gefitinib-based treatment. Lazzari et al. [36] confirmed superior prognosis for TA-discerned good-outcome classification in 111 previously described patients [21]. During treatment, 88% of patients maintained their baseline classification, whereas at treatment withdrawal, largely due to PD, only 26% of good-outcome patients shifted to poor outcome classification [36]. Garrisi et al. identified 7 of 8 TA peaks from spectra of 11 NSCLC patients prior to gefitinib and 10 HCs. Of these, five could be attributed to isoforms of the acute-phase protein serum amyloid A (SAA), but SAA serum concentrations determined by enzyme-linked immunosorbent assay were not related to TTP [37]. From profiles of 34 patients treated with gefitinib plus rofecoxib, O'Byrne et al. found 55 differential peaks between responders ($n = 3$) and patients with SD/PD ($n = 31$) and 90 between patients with disease control ($n = 14$) and nonresponders [38].

Targeted Therapy in Tumors Other Than NSCLC

Hypothesizing that the TA would reflect EGFR dependency regardless of the primary tumor site or the EGFR-targeting agent used, Chung et al. profiled patients with advanced head and neck squamous cell carcinoma (HNSCC; 55 treated with gefitinib [65], 32 treated with erl/bev [66], 21 treated with cetuximab, and 34 treated with docetaxel-based chemotherapy) and 88 cetuximab-treated patients with colorectal cancer (CRC) [67]. For patients with HNSCC, median OS was superior for those classified as good outcome versus poor outcome treated with gefitinib (36.7 vs. 18.0 weeks; HR: 0.41; $p = .007$) and erl/bev (39.5 vs. 29.1 weeks; HR: 0.20; $p = .02$), whereas no significant difference was found in cetuximab-treated patients (38.3 vs.

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Table 1. Eligible studies reporting MS-based peptidomics

First author, year	Tumor type	*n*	Treatment (study of sample origin)	MS platform	Summary results
Targeted therapy					
Taguchi, 2007 [21]	NSCLC	460[a]	Gefitinib, erlotinib [23], none	M	8-peak signature (TA) shows superior TTP and OS for TA-classified good-outcome vs. poor-outcome patients in training and validation sets but not in control set
Salmon, 2009 [31]	NSCLC	180	Erlotinib-bevacuzimab [27], erlotinib [23], chemotherapy	M	11-peak signature (5 overlapping with TA) separates TKI-treated patients with long vs. short PFS and OS
Amann, 2010 [24]	NSCLC	88	Erlotinib [23]	M	Confirmed Taguchi results, independent of KRAS status
Carbone, 2010 [28]	NSCLC	35	Erlotinib-bevacuzimab [27]	M	Superior PFS and OS for TA-classified good-outcome vs. poor-outcome patients
Lazzari, 2012 [36]	NSCLC	111[b]	Gefitinib (overlap with Taguchi training and validation set)	M	Superior PFS and OS for TA-classified good-outcome vs. poor-outcome patients
Kuiper 2012 [32]	NSCLC	50	Erlotinib-sorafenib [33]	M	Superior PFS and OS for TA-classified good-outcome vs. poor-outcome; 9 of 10 responses in TA-classified good-outcome patients
Carbone, 2012 [26]	NSCLC	436[b]	Erlotinib vs. placebo [25]	M	Superior PFS and OS for TA-classified good-outcome vs. poor-outcome placebo patients; superior OS and response rate for TA-classified good-outcome erlotinib vs. placebo patients
Gautschi, 2013 [29]	NSCLC	117	Erlotinib-bevacuzimab [29]	M	Superior OS for TA-classified good-outcome vs. poor-outcome patients
Dingemans, 2013 [34]	NSCLC	57	Sorafenib	M	Superior PFS for TA-classified good-outcome vs. poor-outcome patients
Akerley, 2013 [30]	NSCLC	42	Erlotinib-bevacuzimab	M	Superior PFS and OS for TA-classified good-outcome vs. poor-outcome patients
Stinchcombe, 2013 [35]	NSCLC	98	Gemcitabine, erlotinib, gemcitabine-erlotinib [64]	M	Superior PFS for TA-classified good-outcome vs. poor-outcome patients in erlotinib arms; significant treatment interaction in monotherapy arms
Garrisi, 2011 [37]	NSCLC	11	Gefitinib	S	Identified 7 of TA peaks; 5 attributed to SAA
O'Byrne, 2007 [38]	NSCLC	34	Gefitinib/rofecoxib	M	55 peaks differential for response
Chung, 2010 [39]	HNSCC, CRC	230[a]	HNSCC: gefitinib [65], cetuximab, erlotinib/bevacizumab [66], docetaxel-based chemotherapy. CRC: cetuximab [67]	M	Superior OS for TA-classified good-outcome vs. poor-outcome EGFR-TKI-treated HNSCC patients; superior PFS for TA-classified good-outcome vs. poor-outcome CRC patients
Matsumoto, 2009 [40]	Breast	24	Trastuzumab	M	1 peak associated with clinical response and PFS; low expression discriminated between PD and non-PD with sensitivity of 75% and specificity of 82%
Dalenc, 2010 [41]	Breast	19	Tipifarnib-tamoxifen	S	No relation between pretreatment peaks and outcome
Chemo- and immunotherapy					
Gonçalves, 2006 [42]	Breast	81	Anthracyclin-based (adjuvant) and locoregional radiotherapy	S	5-year MFS 83% vs. 22% in patients with good vs. poor prognosis based on multiprotein index

(continued)

Table 1. (continued)

First author, year	Tumor type	*n*	Treatment (study of sample origin)	MS platform	Summary results
Gast, 2008 [44]	Breast	63	FEC plus high-dose chemotherapy (adjuvant) [68]	S	1 peak associated with RFS
Gast, 2011 [43]	Breast	82[c]	Similar to Gast 2008 [44]	S, M	3 peak clusters significantly associated with RFS
Høgdall, 2010 [45]	Ovarian	131	Platinum-paclitaxel based (adjuvant) [69]	S	2-protein index significantly correlated with PFS
Mazouni, 2010 [46]	Breast	39	Paclitaxel-FEC with or without trastuzumab (neoadjuvant) [70]	M	2 peaks differential for pathological response
Li, 2011 [47]	Osteosarcoma	27[b]	Cisplatin-doxorubicin; high-dose methotrexate (neoadjuvant)	S	56-peak model predictive of pathological response
Voortman, 2009 [48]	NSCLC	27	Cisplatin-gemcitabine/bortezomib [71]	M	5-peak signature predictive of response; 6-peak signature predictive of PFS
Han, 2010 [49]	NSCLC	93	Cisplatin-docetaxel	S	5-peak model predictive of response
Han, 2012 [50]	SCLC	56	Cisplatin-etoposide	S	2-peak model predictive of response
Helgason, 2010 [52]	CRC	42	Capecitabine-oxaliplatin	S	1 peak differential for response
Yuan, 2012 [51]	CRC	70	FOLFOX or FOLFIRI	S	7-peak model predictive of response to FOLFIRI; 6-peak pattern for FOLFOX
Helgason, 2010 [53]	Gastric	68	Epirubicin-cisplatin-capecitabine	S	No differential peaks for response; 1 peak associated with survival
Walter, 2010 [54]	RCC	41	IL-2-interferon-5-FU	S	7-peak model predictive of clinical benefit
Vermaat, 2010 [55]	RCC	114	Interferon-based, TKIs, other	S	10 proteins related to survival; 2-protein signature significantly predicted survival and improved current risk models
Surgery					
Risum, 2009 [56]	Ovarian	75	Debulking surgery [23]	S	7-peak signature predictive of incomplete cytoreduction
Wood, 2010 [57]	Renal	119	Nephrectomy	S	6 peaks independently associated with CSS
Xue, 2012 [58]	Pancreatic	61	Pancreaticoduodenectomy	S	3-peak model predictive of survival $<$1 year
Radiation and chemoradiation therapy					
Su, 2014 [59]	Nasopharyngeal	50	70–76 Gy	S	11 differential peaks; 4-peak model predictive of radiosensitivity
Hayashida, 2005 [61]	Esophageal	42	40 Gy with 5-FU-cisplatin (neoadjuvant)	S	4-peak signature predictive of pathological response
Maher, 2011 [62]	Esophageal	31	40–44 Gy with 5-FU-cisplatin (neoadjuvant)	S	4 peaks differentially expressed; C3a and C4a expression predictive of pathological response
Kelly, 2012 [63]	Esophageal	24	Chemoradiation (neoadjuvant), palliative chemotherapy, none	S	3 peaks independently associated with 8-month DFS, 8- or 12-month survival
Smith, 2007 [60]	Rectal	20	45 Gy with 5-FU	S	14-peak signature after 24–48 hours of treatment predictive of pathological response

Profiles based on serum unless otherwise specified.
[a]Serum/plasma.
[b]Plasma.
[c]Fractionated serum.
Abbreviations: 5-FU, 5-fluorouracil; CRC, colorectal cancer; CSS, cancer-specific survival; DFS, disease-free survival; FEC, 5-FU, epirubicin and cyclophosphamide; FOLFIRI, 5-FU and irinotecan; FOLFOX, 5-FU and oxaliplatin; HNSCC, head and neck squamous cell carcinoma; IL-2, interleukin 2; M, matrix-assisted laser desorption/ionization time-of-flight; MFS, metastasis-free survival; MS, mass spectrometry; NSCLC, non-small cell lung cancer; OS, overall survival; PD, progressive disease; PFS, progression-free survival; RCC, renal cell carcinoma; RFS, recurrence-free survival; S, surface-enhanced laser desorption/ionization time-of-flight; SAA, serum amyloid A; SCLC, small cell lung cancer; TA, Taguchi algorithm; TKI, tyrosince kinase inhibitor.

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11.9 weeks; HR: 0.26; $p = .06$) and chemotherapy-treated patients (39.4 vs. 15.5 weeks; HR: 0.88; $p = .76$). OS data were not available for the CRC patients, but PFS was significantly longer for good-outcome patients versus poor-outcome patients (8.9 vs. 8.4 weeks; HR: 0.51; $p = .007$) due to the observed separation beyond the 9-week response assessment point [39].

Two eligible studies were performed in patients with advanced breast cancer. Aiming to identify glycosylation biomarkers for trastuzumab, Matsumoto et al. analyzed plasma N-glycan profiles of 24 patients. One peak was significantly lower in patients with PD [40]. From profiles of 19 patients treated with tamoxifen plus tipifarnib, a farnesyltransferase inhibitor, Dalenc et al. identified 1 peptide that was significantly associated with TTP in serum obtained after 8 weeks of treatment, whereas pretreatment sera did not provide such association [41].

Studies Reporting Profiling Prior to Chemo- or Immunotherapy

Four eligible studies performed postoperative profiling prior to adjuvant chemotherapy in three in patients with breast cancer and one patient with ovarian cancer [42–45]. Although the candidate biomarkers identified in these studies most likely have prognostic relevance, a relationship to chemosensitivity cannot be excluded.

Mazouni et al. evaluated profiles from 39 patients with HER2-positive breast cancer receiving neoadjuvant paclitaxel-anthracyclin-based chemotherapy [70]. Two peptides were differentially expressed in patients with pathological complete response compared with patients with residual disease [46]. Plasma profiles of 27 osteosarcoma patients treated with preoperative cisplatin-doxorubicin and high-dose methotrexate have been investigated by Li et al., who considered patients with tumor necrosis of 90% or higher in the resection specimen as responders. The resulting 56-peak model predicted response with overall accuracy of 85% [47].

Two studies reported profiling prior to palliative chemotherapy in patients with NSCLC. From patterns of 27 previously untreated patients treated with cisplatin-gemcitabine plus the proteasome inhibitor bortezomib [71], Voortman et al. identified 6 differential peptides based on relatively short versus long PFS in the most distinct 22 patients. When applied to all samples, the signature showed a significantly shorter median PFS in patients classified as having short versus predicted long PFS (120 vs. 191 days; $p = .036$). Median OS of these patients was also significantly different at 144 versus 436 days ($p = .036$). Five differential peaks were identified between patients responding with PR ($n = 9$) and non-PR, of which two overlapped with the previous signature. This signature revealed a significantly shorter PFS and a nonsignificantly shorter OS for patients with non-PR [48]. Han et al. investigated profiles of 93 patients treated with first-line cisplatin-docetaxel chemotherapy. Considering only patients with PR as chemotherapy-sensitive, 76% of patients showed initial resistance. From a training set of 62 patients, a 5-peptide model was constructed that accurately separated chemotherapy-resistant patients from chemotherapy-sensitive patients in 84% of the blinded test set ($n = 31$) [49]. A similar approach by the same investigators in 56 SCLC patients treated with cisplatin-etoposide resulted in a 2-peak model performing with 80% accuracy [50].

Two eligible studies performed profiling prior to first-line palliative chemotherapy in patients with advanced CRC. Yuan et al. analyzed sera of 70 patients treated with 5-fluorouracil (5-FU) and oxaliplatin (FOLFOX, $n = 44$) and 5-FU and irinotecan (FOLFIRI, $n = 26$). Regarding patients with PR or SD as responders, a signature of the six most discriminating peaks for FOLFOX and the seven (nonoverlapping) peaks for FOLFIRI was constructed. Their performance in a randomly chosen test set is summarized in Table 2 [51]. Helgason et al. identified 1 differential peptide in profiles of "polar opposite" responders (20 PR, 10 PD) out of 40 patients treated with capecitabine-oxaliplatin [52]. A similar approach in 68 patients with advanced gastric cancer eligible for response evaluation upon treatment with first-line epirubicin, cisplatin, and capecitabine did not result in differential peaks, but low intensity of 1 peptide (11,600 Da, SAA) was significantly correlated to longer OS [53].

Since the introduction of targeted agents, immunotherapy has been decreasingly applied in metastatic renal cell carcinoma (mRCC), despite its ability to induce durable responses. Walter et al. investigated serum profiles of 41 patients treated with interleukin-2, interferon, and 5-FU. Constructed from a pattern of 25 proteins associated with response, including SAA isoforms and transthyretin (TTR), a rule base could classify patients as having predicted clinical benefit versus PD with accuracy of 66% [54]. Vermaat et al. identified 10 proteins significantly associated with OS in serum from 114 patients with mRCC, with 73% obtained prior to first-line interferon-based treatment. Serum concentrations of identified proteins apolipoprotein-A2 (Apo-A2), SAA, and TTR were predictive of survival; SAA and TTR levels were able to improve the commonly used prognostic mRCC model [55].

Studies Reporting Profiling Prior to Local and Combination Treatment

Surgery

Ultimately, predicted nonresponders to surgery might receive additional adjuvant or neoadjuvant systemic treatment to improve their outcome or palliative treatment only. Risum et al. analyzed preoperative serum profiles and CA-125 levels to predict incomplete primary debulking surgery in 75 patients with stage III–IV ovarian cancer. A panel of 7 of 10 prespecified proteins were combined into a single-valued ovarian cancer risk index (OvaRI). Overall accuracy was 72% for the OvaRI and 67% for CA-125, but combined analysis did not improve the predictive power of either analysis [56]. Wood et al. identified 6 peaks independently associated with cancer-specific survival but not with DFS after nephrectomy in serum of 119 patients with RCC; one peak (1,525 Da) was identified as an SAA fragment [57]. From 40 patients with pancreatic cancer who planned to undergo pancreaticoduodenectomy, eventually followed by adjuvant therapy in 87.5% of patients, Xue et al. developed a 3-peptide panel that, when combined with CA 19-9, predicted survival of less than 1 year with an area under the receiver operating characteristic curve of 0.96. In the verification set of 21 patients, predictive accuracy was 76%. One peptide was identified as ApoC-II, for which serum levels significantly correlated to short survival in an independent validation set [58].

Table 2. MS-based classifier characteristics

First author, year	MS-based classification model	Peptide peaks (m/z)	Independent validation / test set	Prediction accuracy (%)	Sensitivity (%)	Specificity (%)
Gonçalves, 2006 [42]	40-peak based	Including 8,936 (C3a), 9,192 (Hp I), 81,763 (transferrin), 28,284 (Apo-A1), 6,647 (Apo-C1)	—	72	73	70
Han, 2010 [49]	5-peak	3,955, 6,207, 7,992, 9,214, 15,086	+	84	83	86
Han, 2012 [50]	2-peak	8,830, 10,468 (S100-A9)	+	80	80	80
Hayashida, 2005 [61]	4-peak	7,420, 9,112, 12,867, 17,123	+	93	100	80
Li, 2011 [47]	56-peak	Including 1,1467, **11,530** (SAA)	—	85	81	91
Risum, 2009 [56]	7-peak	Including 3,272, 12,828, 28,043 (Apo-A1)	—	72	73	70
Salmon, 2009 [31]	11-peak	**4,121**, 4,596, 4,720, 4,821, 5,720, 5,841, **11,441**, **11,528**, **11,684**, **11,731**, **11,902** (SAA)	+	NS	NS	NS
Smith, 2007 [60]	14-peak	2,079, 2,093, 2,131, 2,159, 2,338, 2,524, 3,049, 3,150, 4,159, 4,188, 5,856, 7,042, 9,056, 15,339	—	NS	55	64
Su, 2014 [59]	4-peak	2,575, 3,942, 6,117, 6,778	—	78	85	71
Taguchi, 2007 [21]	8-peak	5,843, **11,446, 11,530, 11,685, 11,759, 11,903** (SAA), 12,452, 12,580	+	NS	NS	NS
Voortman, 2009 [48]	6-peak	1,545, 2,209, **2,215**, **2,318**, 2,376, 2,489	—	82	82	82
Voortman, 2009 [48]	5-peak	900, 2,009, **2,215**, **2,318**, 2,378	—	89	100	83
Walter, 2010 [54]	7-peak	4,145, 5,715, **11,479** (SAA), 40,412, 51,203, 133,146, 185,034	—	66	NS	NS
Xue, 2012 [58]	3-peak + Ca19.9	3,700, 8,222 (Apo-C2), **11,522** (SAA-I)	+	76	55	100
Yuan, 2012 [51]	6-peak	2,266, 4,606, 7,775, 9,198, 9,282, 9,298	—	NS	93	81
Yuan, 2012 [51]	7-peak	2,648, 2,952, 3,980, **4,121**, 4,292, 4,305, 4,321	—	NS	92	92

Reported classification models with discriminatory peaks and accuracy parameters. Prediction accuracy equals the proportion correct among the total number of predictions. Bold text indicates peaks that are present in multiple models.
Abbreviations: +/−, indicates the presence or absence of an independent data set, included for validation of the classification model; Apo, apolipoprotein; MS, mass spectrometry; m/z = mass-to-charge ratio; NS = not specified; SAA, serum amyloid A.

Radiation and Chemoradiation Therapy

Su et al. analyzed serum profiles of 50 patients with nasopharyngeal cancer in relation to response to 7–8 weeks of radiation therapy, as assessed by nasopharyngoscopy and computed tomography scanning. Only patients exhibiting rapid regression were considered radiation sensitive. A 4-peptide pattern derived from 11 differential peaks predicted radiosensitivity in 78% of patients [59].

Three eligible studies evaluated profiling in relation to pathological response to neoadjuvant 5-FU-based chemoradiation [60–62]. Smith et al. analyzed sera taken before and during course of treatment from 20 patients with rectal cancer in relation to response according to the Mandard tumor regression grade (TRG). Although the "optimal" pretreatment model performed poorly, a 14-peptide classifier in serum taken 24–48-hours after treatment resulted in sensitivity of 88% and specificity of 80% [60]. Applying an alternative 3-grade scale for response, Hayashida et al. constructed a 4-peptide signature from profiles of 27 patients with squamous cell esophageal cancer. This performed with 93% accuracy in an independent validation set ($n = 15$) at sensitivity of 100% and specificity of 80% [61]. In 31 patients with mixed histological-type esophageal cancer, Maher et al. identified 4 differential peptides between TRG-based responders and nonresponders; 2 were identified as complement C3a and C4a. Their baseline serum levels predicted response witrun -1th sensitivity of 79% and specificity of 83% in 87% of patients [62]. From profiles of 24 patients with esophageal cancer, for whom treatment included neoadjuvant chemoradiation ($n = 11$) and palliative chemotherapy ($n = 8$), Kelly et al. identified TTR (14,029 Da), Apo-A1 (27,665 Da) and SAA (11,670 Da) as independently associated with 8-month DFS and survival at 8 and 12 months, respectively [63].

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Table 3. Outcome of non-small cell lung cancer patients in pooled analysis

		Outcome according to classification			
		Poor outcome		Good outcome	
First author, data set	Cohort or study arm, *n*	Median TTP/PFS	Median OS	Median TTP/PFS	Median OS
Taguchi, 2007, training set [21]	Gefitinib, 139	2.1	4.9	5.3	14.5
Taguchi, 2007, validation set 1 [21]	Gefitinib, 67	2.0	3.0	2.8	6.8
Taguchi, 2007, validation set 2 [21]	Erlotinib, 96	1.9	3.5	3.2	10.1
Carbone, 2010 [28]	Erlotinib-bevacizumab, 35	1.8	5.5	8.3	14.0
Kuiper, 2012 [32]	Erlotinib-sorafenib, 50	2.7	5.6	5.5	13.7
Carbone, 2012 [26]	Erlotinib, 292	1.8	4.0	3.7	10.5
Gautschi, 2013 [29]	Erlotinib-bevacizumab, 117	3.2	6.2	4.0	13.4
Dingemans, 2013 [34]	Sorafenib, 57	1.5	2.5	2.6	6.0
Akerley, 2013 [30]	Erlotinib-bevacizumab, 42	1.4	4.6	4.3	16.4
Stinchcombe, 2013 [35]	Erlotinib, 38	0.7	1.7	2.9	8.4
Stinchcombe, 2013 [35]	Erlotinib-gemcitabine, 32	2.9	3.5	4.0	9.9
Stinchcombe, 2013 [35]	Gemcitabine, 28	4.5	6.5	4.4	6.6
Voortman, 2009 [48]	Gemcitabine-cisplatin-bortezomib, 27	3.9	4.7	6.3	14.3

Outcome in months for 1,020 treated patients with advanced non-small cell lung cancer, available from 9 studies with nonoverlapping data sets. Approximately 49% of patients did not receive prior treatment. Reported outcome duration in days was divided by 30.43 to calculate duration in months.
Abbreviations: TTP, time to progression; OS, overall survival; PFS, progression-free survival.

MS-Based Classification Models

Fourteen studies developed an MS-based classification model. Differential peptide peaks from these models and their performance in terms of overall accuracy, sensitivity, and specificity are summarized in Table 2. Median accuracy was 81% (range: 66%–93%) in 11 of 14 studies reporting performance of the classifier. Additional mass peaks associated with outcome that were not validated or applied into an MS-based prediction model are listed in supplemental online Table 1.

Pooled Analysis of Survival Times in NSCLC Studies

As an example of the potential power of serum and plasma peptidomics for prediction of treatment outcome in NSCLC, we pooled TTP/PFS and OS times available for the treated patients (933 received targeted therapy, 87 received chemotherapy-based treatment) from 9 discussed NSCLC studies (Table 3). This analysis revealed median PFS and OS in poor-outcome patients of 2.00 $\pm$ 1.06 and 4.58 $\pm$ 1.45 months, respectively, whereas in good-outcome patients, median PFS was 4.01 $\pm$ 1.60 months and median OS was 10.52 $\pm$ 3.49 months (Fig. 4). Aware of the small number of chemotherapy-treated patients, we also specified outcome for treatment type by algorithm status. PFS for poor-outcome patients was significantly shorter for those treated with targeted therapy than for chemotherapy-treated patients (1.87 vs. 3.94 months, $p = .002$), whereas OS was not different (4.28 vs. 4.73 months, $p = .459$). For patients classified as good outcome treated with targeted therapy and chemotherapy, median PFS and OS did not differ (PFS: 3.85 vs. 4.37 months; $p = .579$; OS: 9.92 vs. 11.98 months; $p = .651$).

Discussion

We have systematically reviewed available literature on pretreatment MS-based serum and plasma peptidomics in adult patients with solid malignancies in relation to treatment outcome to evaluate classification accuracy and readiness of this approach for clinical implementation.

> Together, these results support the proof-of-concept for MS-based pretreatment profiling to influence clinical decision making in NSCLC, for example, by preferential chemotherapy for TA-classified poor-outcome patients, as has been suggested previously.

Thirteen of 38 included studies investigated profiling prior to targeted therapy in patients with advanced NSCLC. These were mostly related to a study published in 2007 by Taguchi et al. [21] in which an eight-peptide signature indicative of PFS and OS in patients treated with EGFR TKIs was identified. Five of these peaks have been identified as isoforms of the acute-phase protein SAA [32, 37, 72]. Several studies confirmed the prognostic properties of this algorithm in patients treated with targeted therapy-containing regimens [26, 30, 32], and some also indicated predictive potential [21, 35, 39]. We performed a pooled analysis of TTP/PFS and OS times available from 9 NSCLC studies. This revealed clinically significant median PFS and OS in poor-outcome patients, indicative of the potential of blood-based peptidomics, for consideration of withholding treatment in these patients (Fig. 4). In addition, the longer PFS for chemotherapy versus targeted therapy in patients classified as poor outcome (3.94 vs. 1.87 months; $p = .002$) in this exploratory analysis may point in the same predictive direction as the results of the recently presented PROSE study [73]. In this TA-stratified study, 285 patients with advanced NSCLC were randomized between second-line treatment with erlotinib or chemotherapy. In patients classified as poor outcome, OS was significantly shorter for those treated with erlotinib than for chemotherapy-treated

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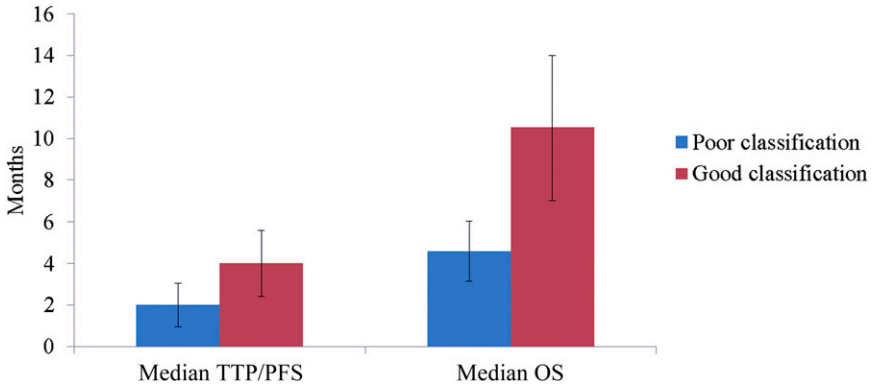

Figure 4. Pooled analysis of non-small cell lung cancer studies. Pooled PFS and OS analysis according to pretreatment mass spectrometry-based classification of patients reported in Table 3.

Abbreviations: OS, overall survival; PFS, progression-free survival; TTP, time to progression.

patients (3.0 vs. 6.4 months; HR: 1.72; $p = .022$), whereas no difference was found in patients classified as good outcome (11.0 vs. 10.9 months; HR: 1.06; $p = .714$). Together, these results support the proof-of-concept for MS-based pretreatment profiling to influence clinical decision making in NSCLC, for example, by preferential chemotherapy for TA-classified poor-outcome patients, as has been suggested previously [73, 74].

Promising results of pretreatment MS-based peptidomics have also been obtained in other tumor types and treatment modalities, including neoadjuvant treatment for osteosarcoma and esophageal cancer, with prediction accuracies of the reported classifiers of 85% and 93%, respectively [47, 61].

Limitations of the reviewed studies included small sample sizes (median $n = 59$), variable design and outcome parameters, and absence of independent validation sets in most studies. In fact, validation of the signature was attempted in an independent cohort in only 6 of 14 studies reporting construction of an MS-based prediction model based on differential peaks (Table 2). One of our concerns that we were not able to address in detail in this review regards the quality of the applied data analysis methods and algorithm construction. Variability in sample collection and preparation may be responsible for the lack of peak reproducibility in studies investigating similar tumor types (Table 2). In recent years it has become clear that the use of a serum pool as a control and strict handling rules are crucial because several preanalytical factors, including clotting time and temperature, may influence reproducibility [75, 76]. The majority of studies did not provide details on sample handling and analysis and did not report the use of a prespecified collection protocol. In addition, they did not provide information on the exact interval between serum or plasma collection and treatment initiation.

In the discussed studies, SAA and apolipoprotein were the most frequently identified proteins in patients with a variety of tumors and treatments. It is known that patients with more advanced disease or poorer prognosis have increased blood levels of these markers.

Ideally, the identity or "source protein" of peptides constituting an MS-based blood signature would be known. This may enhance understanding of the underlying tumor biology and would facilitate validation and clinical implementation of these biomarkers. It is important to realize that a large part of the serum peptides are degradation products from circulating nontumor-derived proteins, resulting from differential protease activity in the tumor microenvironment [17], which may even involve or reflect an acute-phase host response [32]. Some of the peptides may also be generated from ex vivo protease activity during the clotting process required for serum preparation in the tube on venipuncture [17]. In the discussed studies, SAA and apolipoprotein were the most frequently identified proteins in patients with a variety of tumors and treatments. It is known that patients with more advanced disease or poorer prognosis have increased blood levels of these markers [55, 77, 78]. Consequently, corresponding mass peaks of these proteins are most likely prognostic markers. Alternative blood-based methods are in development, including genomics-, transcriptomics- and phosphoproteomics-based profiling [79–81], but investigation is required to determine their advantages over serum and plasma peptidomics in terms of predictive value and accuracy.

Conclusion

Pretreatment MS-based serum and plasma peptidomics have shown promising results for prediction of treatment outcome in patients with solid tumors. Limited sample size and lack of signature validation in most studies have prohibited clinical implementation thus far, despite although the technology within the field has matured. Our pooled analysis and results from the PROSE study indicate that this blood-based profiling approach enables treatment selection in patients with cancer, but additional prospective studies are warranted. Future studies should be designed to facilitate clinical decision making. They should have sufficient sample sizes and encompass uniform and strict sample collection and handling protocols and include validation efforts for the identified putative biomarkers using patient cohorts that are independent, from multiple centers, and preferably from multiple countries. Moreover, consensus on criteria to evaluate clinical implementation of proposed treatment selection tools is needed.

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